For the **experiment**, mice were genotyped and hippocampii were dissected from 3 mutant and 3 wild-type animals. Total RNA was extracted using Trizol and samples were purified with RNeasy™ Kit (Qiagen). Sequencing libraries were prepared after PolyA RNA isolation using mRNA-sequencing kit (Illumina).

For **mapping**, Raw sequence reads were mapped back to the mouse reference genome (mm9) together with a comprehensive database of annotated exon junctions compiled from mouse, human, and rat mRNA/EST data.

The program tophat (v1.3.2) with bowtie (v3.4.6) was used for alignment (parameters: -a 5 -F 0.05 -r 150 -j <junction.hmr.raw>)

Paired ends could be located in different exons and since the reads are relatively short, the transcript structure between the sequenced ends could be ambiguous when alternative splicing occurs. The missing information, leveraging on the size constraints of each cDNA fragment, was inferred using a simple Bayesian model. Specifically, the distribution of fragment size was estimated using paired-end reads located in the same exons. Large exons (≥ 400 nt) were used for this purpose to avoid bias, since the rough estimate of the average fragment size is ~130 nt. In parallel, the number of observed junction reads for each exon junction was counted. During statistical inference, for each pair of reads that spans one or more exons (up to three, which is sufficient in practice given the fragment size), we enumerated all possible isoforms (paths) P_j between the

anchored ends, and estimated the probability of each isoform to be the actual origin of the paired-end reads, using the Bayes formula:

$$\Pr(P_k \mid l) = \frac{\Pr(l \mid P_k)\Pr(P_k)}{\sum_j \Pr(l \mid P_j)\Pr(P_j)} = \frac{\Pr(l_k)\Pr(P_k)}{\sum_j \Pr(l_j)\Pr(P_j)}$$

where l_j is the length of the fragment if the reads were derived from isoform j; $\Pr(l_j)$ and $\Pr(P_j)$ are the distribution of fragment size and prior distribution of each isoform, respectively. Following analyses using inferred fragments, each was assigned a probability score. This junction inference step substantially increased the effective number of fragments supporting exon junctions, especially for cassette exons, and increased statistical power in detecting splicing changes. We then counted the weighted number of exon or exon-junction fragments uniquely supporting the inclusion or skipping isoform of each cassette exon. Biological replicates of each group were pooled together. Additional filtering criteria on junction fragment coverage were applied to reduce the multiple testing (junction_in + junction_skip≥20, junction_WT+junction_KO≥20; 2,149 cassette exons remained). A Fisher's exact test was used to evaluate the statistical significance of splicing changes using both exon and exon-junction fragments, followed by Benjamini multiple testing correction to estimate the false discovery rate (FDR). In addition, inclusion or exclusion junction reads were used to calculate the proportional change of exon inclusion (ΔI). Differential splicing events were identified by requiring FDR<0.15, or more stringent criteria (FDR <0.05 and |ΔI| ≥0.1), which defined a subset with high confidence (Table S1).